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## Taxonomic Study of the Kinorhyncha in Japan

### I. *Pycnophyes tubuliferus* Adrianov, 1989 (Kinorhyncha: Homalorhagida) from Japan

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**Abstract** A pycnophyid kinorhynch, *Pycnophyes tubuliferus* Adrianov, 1989, collected from muddy sediment at Tanabe Bay, Honshu Island, Japan is re-described and illustrated. This is the first record of the order Homalorhagida and the genus *Pycnophyes* from Japanese waters, and the fourth genus of the class Kinorhyncha from Japan. Previously *P. tubuliferus* was found only in a few localities alongside the Russian and Korean coasts in the western part of the Sea of Japan, and this is the first finding of this species outside the Sea of Japan. Detailed morphology of the species has been studied using Nomarsky microscopy and SEM for the first time. Basic taxonomic characters were compared from zoogeographic points of view among specimens representing populations from the Sea of Japan (including type-material) and the Pacific coast of the mainland of Japan. The new diagnosis of the species has been proposed.

**Key words:** kinorhynch, placid, flosculus, tubulus, sensory spot, pachycyclus, *Pycnophyes tubuliferus*, redescription, taxonomy

## Introduction

Kinorhyncha constitute a taxon of meiobenthic, free-living, segmented and spined marine invertebrates, generally less than 1 mm in length. Previously, the taxon has been considered as a class of the phylum Aschelminthes (see Hyman, 1951), but currently is considered an independent phylum with close relationships to aschelminth worms (see Higgins, 1971; Kristensen and Higgins, 1991). Recently, the Kinorhyncha has been included as a class in the phylum Cephalorhyncha (see Adrianov and Malakhov, 1994, 1999).

In contrast to several publications on the cephalorhynch taxa from the continental coast of the North-West Pacific (see Adrianov, 1989; Adrianov and Malakhov, 1994, 1996, 1999), information on the kinorhynch fauna from Japanese Islands is fragmental. A few studies have reported presence of kinorhynchs (otherwise unidentified) from Japanese waters, and most of them were dealing with ecology of subtidal meiofauna. The first report of Kinorhyncha from Japan was *Echinoderes* sp. (Kawamura, 1927) collected by Komai in 1925 at Misaki Marine Biological Station near Tokyo (see Kawamura, 1927, 1947; Tokioka, 1949). Years later, Abe (1930) described a new species, *Echinoderes masudai* Abe, 1930. It is a cyclorhagid kinorhynch found at Gogoshima Island near Hiroshima. The description of the species was however too poor to do comparisons practically, and *E. masudai* is not currently designated as a valid species and considered as a “species indeterminata” (Higgins, 1983; Adrianov and Malakhov, 1999). In 1949, Tokioka reported another species of *Echinoderes*, *E. dujardinii* Claparède, 1863, from Ago Bay, Honshu Is. (Tokioka, 1949). Some years later, this species was listed by Sudzuki (1976 a, b) from meiobenthic samples around Kasado Island in the Seto Inland Sea of Japan. The finding of *E. dujardinii* in Japan is however highly questionable because the species has been known only from European waters, and the reports of Tokioka (1949) and Sudzuki (1976 a, b) seem misidentification (see Higgins, 1983; Adrianov and Malakhov, 1994). The unidentified species of the genus *Trachydemus* (= *Kinorhynchus*) has been

reported and illustrated by Sudzuki (1976 a) from the station between Kasado and Honshu Islands., the Seto Inland Sea of Japan. The generic name "*Trachydemus* Zelinka, 1907" is preoccupied by *Trachydemus* Chevrolat, 1873 (Coleoptera) and it has been replaced by the name *Kinorhynchus* Sheremetevsky, 1974. Because the prominent autapomorphic characters were well illustrated by Sudzuki (1976 a), this species is conspecific with *Kinorhynchus yushini* Adrianov, 1989, previously described from the Peter the Great Bay, north Sea of Japan, and found also from the coast of Korea (see Adrianov, 1989; Adrianov and Malakhov, 1999). In 1979, Sudzuki also reported unidentified species of *Echinoderes* in meiofaunal samples taken from intertidal zones of Iriomote and Okinawa Islands, Ryukyu, southwest Japan. More recently, Higgins and Shirayama (1990) described a new species, *Dracoderes abei* Higgins et Shirayama, 1990, with erection of a new genus and a new family of the Kinorhyncha. This species was found in the Mukaishima yacht harbor, southwest of the Mukaishima Marine Biological Station in the Seto Inland Sea of Japan (Higgins and Shirayama, 1990).

Representatives of the genus *Pycnophyes* have never been reported in Japan. The purpose of this paper is to compare the taxonomic attributes of the Japanese specimens with those of the type-material. The species was identified as *P. tubuliferus*, previously described by Adrianov (1989) from the Peter the Great Bay, north Sea of Japan, and found from Korean coasts too (Adrianov and Malakhov, 1999). The first description of *P. tubuliferus* is however too poor to do comparisons practically. The species was redescribed by Adrianov in 1999 (see Adrianov and Malakhov, 1999) using Nomarsky optics, with new drawings of specimens from the type material. Though the species already studied well in light microscopy, detailed morphological study has never been done. We carried out observation of the species using both Nomarsky light microscopy and scanning electron microscopy, and a new finding based on this observation also will be described.

### Materials and Methods

The specimens used in this study were collected in 1999–2000 from mud sample taken by a meiobenthic dredge at a depth of 13–25 m in the Tanabe Bay, located at Kii peninsula of Honshu Island (33°42.2' N and 135°22.9' E) in the vicinity of the Seto Marine Biological Laboratory, Kyoto University (Fig. 1). Living kinorhynchs were extracted from the sediment by the "bubble-and-blot" technique (Higgins, 1983). Most specimens were fixed in 10% buffered formalin in seawater. Some of them were transferred into a 70 % ethanol –5% glycerol –25% deionized water solution. Letting ethanol and water evaporate, the material was preserved in anhydrous glycerol. About 65 specimens then were mounted individually in Hoyer's-125 mounting medium between two cover slips, and positioned on Cobb aluminum or Higgins-Shirayama plastic slide frames for further examination using differential interference contrast optics (Nomarsky optics).

About 30 specimens were selected for scanning electron microscopy (SEM). These specimens were transferred by an Irwin Loop from 10% formalin to a vessel of distilled water and washed using a detergent to clean the body surface. The cleaned specimens were transferred to a minimal volume of distilled water and ethanol was added slowly until the concentration became close to 100 percent. Thereafter, the specimens were washed by absolute ethanol several times, and finally replaced by tertiary-butyl alcohol. The specimens were dried in a freeze dryer, mounted on stabs, coated with Platinum-Paladium, and observed using a SEM (Hitachi S-4300).

To carry out fine morphological comparison of *P. tubuliferus* among populations from the western Sea of Japan and the Pacific coast of mainland of Japan, about 25 specimens from Korea (Ulsan Bay, 35°27.50' N and 129°23.62' E) were also examined using the same method (10 for Nomarsky, 15 for SEM). The type material (holotype-Ic 278; topotypes AVA-910525.1 (5)-adult female and AVA-910525.4 (2)-adult male) was also re-examined with Nomarsky optics. Good-quality Nomarsky photographs of specimens of *P. tubuliferus* from the type locality and from Korea, previously published in Adrianov and Malakhov (1999, pp. 207–208) were also analysed.

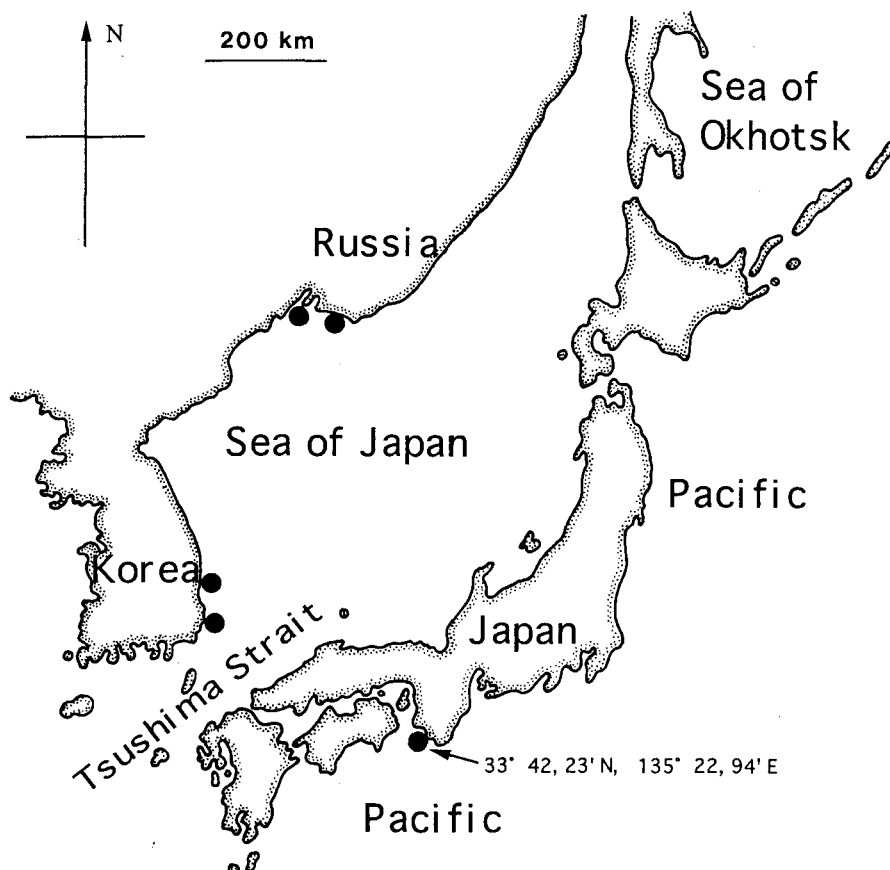


Fig. 1. Map showing the distribution of *Pycnophyes tubuliferus* in the North-West Pacific. Black spots indicate separate localities.

In the examination procedures, we followed the standard protocol described by Higgins (1983). Measurements are given in micrometers. Ratios are expressed in percent of the total length (TL) measured on the midline, from the anterior margin of segment 3 (first trunk segment) to the posterior margin of segment 13, exclusive of spines. Maximum sternal width (MSW) is measured at the anteroventral margin of the widest pair of sternal plates as first encountered in measuring each segment from anterior to posterior. Standard width (SW), or sternal width of segment 12, is measured at the anteroventral margin of 12th sternal plates. Measurements are given for length of trunk segments (L), lateral terminal spines (LTS) and penile spines of male (PS). The locality data from material examined are referred to the collector's initials (AVA and CM). The specimens in the code CM have been deposited in the meiofaunal collection of the Seto Marine Biological Laboratory of Kyoto University, and those in AVA in the meiofaunal collection of the Institute of Marine Biology in Vladivostok, Russia.

Abbreviations used in the text, figures and table are as follows:

AP-middorsal anterior projection; AT-adhesive tube; CI-cilium; CR-cuticular ridge; ESP-episternal plate; HP-horn-like protrusion; L-length of segment; LP-lateral plate (= lateral articulation); LTS-lateral terminal spine; MD-middorsal element of segments 3-13; MP-middorsal protrusion; MS-muscular scar; MSP-midsternal plate; MSW-maximum midsternal width; MT- antero-mesial thickenings of ventral pachycycli ("Mittelwuelste"); PA-pachycyclus; PE-petals; PL-placid;

PS—penile spine; RC—area of reticulate cuticle; S—segments 1–13; SE—seta; SS—sensory spot; SW—standard width; T—tergal plates of segments 3–13; TC—thin cuticle area; TL—trunk length; TP—tubular papilla with floscular appearance.

### Results

Based on interference contrast and SEM investigation we would like to provide a standardized description of female and male characters of *P. tubuliferus* with a standard table of mean measurements for 5 representatives of each sex.

#### Taxonomic account

Order Homalorhagida (Zelinka, 1896) Chitwood, 1958  
Suborder Homalorhagae Zelinka, 1896  
Family Pycnophyidae Zelinka, 1896  
Genus *Pycnophyes* Zelinka, 1896  
*Pycnophyes tubuliferus* Adrianov, 1989  
(Figs. 2–7)

New locality. The Tanabe Bay, Kii Peninsula, Honshu Is., Japan, Northwest Pacific (33°42.23' N, 135°22.94' E); grey mud, 13–14 m depth (Fig. 1).

Description. (1) Adult female (AVA JAP-1.2) (Fig. 2 A–B)—TL 640  $\mu\text{m}$ ; MSW-7 134  $\mu\text{m}$ , 20.9% of TL; SW 106  $\mu\text{m}$ , 16.6% of TL; LTS 154  $\mu\text{m}$ , 24.1% of TL.

Segment 1. Head withdrawn.

Segment 2. Neck consisting of 4 dorsal placids with concave anterior margin and 2 ventral placids with even anterior margin. Cuticular fold between segments 2 and 3 with two sharp horns protruding laterally of anterior margin of midsternal plate of segment 3.

Segment 3. 1st trunk segment; L-3 97  $\mu\text{m}$ ; anterior tergal margin slightly wavy conforming to dorsal placids, with wide submarginal area of reticulate cuticle; width at anterior margin 126  $\mu\text{m}$ ; horn-like lateral projections well developed, long, slightly blunt; tergal plate with a pair of subdorsal cuticular scars, a pair of subdorsal sensory spots posterior to cuticular scars, a pair of dorsolateral muscular scars, a pair of dorsolateral sensory spots, and a pair of dorsolateral seta laterally to dorsolateral sensory spot; midsternal plate trapezoidal, 80  $\mu\text{m}$  long, width of anterior margin 32, width of posterior margin 42  $\mu\text{m}$ ; midsternal plate with oval-shaped area of thin cuticle with two separate spots; anterior margin of midsternal plate slightly protruding beyond anteromesial margins of episternal plate; episternal plate with large anteromesial areas of thin cuticle with reticulate cuticular microrelief; each episternal plate with cuticular scar, ventrolateral seta and sensory spot near thin cuticle area.

Segment 4. L-4 54  $\mu\text{m}$ ; MSW-4 128  $\mu\text{m}$ ; tergal plate with minute middorsal element, never protruding beyond posterior margin of tergite; tergal plate with middorsal seta, a pair of middorsal sensory spots near middorsal element, a pair of subdorsal cuticular scars, a pair of subdorsal sensory spots, and two pairs of dorsolateral setae; anterior margin of tergal plate with blunt anterior middorsal projection, slightly protruding beyond posterior margin of segment 3; posterior middorsal protrusion underdeveloped and never protruding beyond posterior margin of tergite; with characteristic peg-and-socket tergal-sternal articulation, with thick midventral and anteroventral pachycycli; sternal plates with a pair of cuticular scars, a pair of subventral sensory spots, a pair of subventral setae; posterior margin of sternal plates slightly concave laterally; with a pair of lateral setae on tergal plate next two articulation site; tergal and sternal plates with series of parallel transverse cuticular ridges of minute fringe, extending mesially from lateral margins.

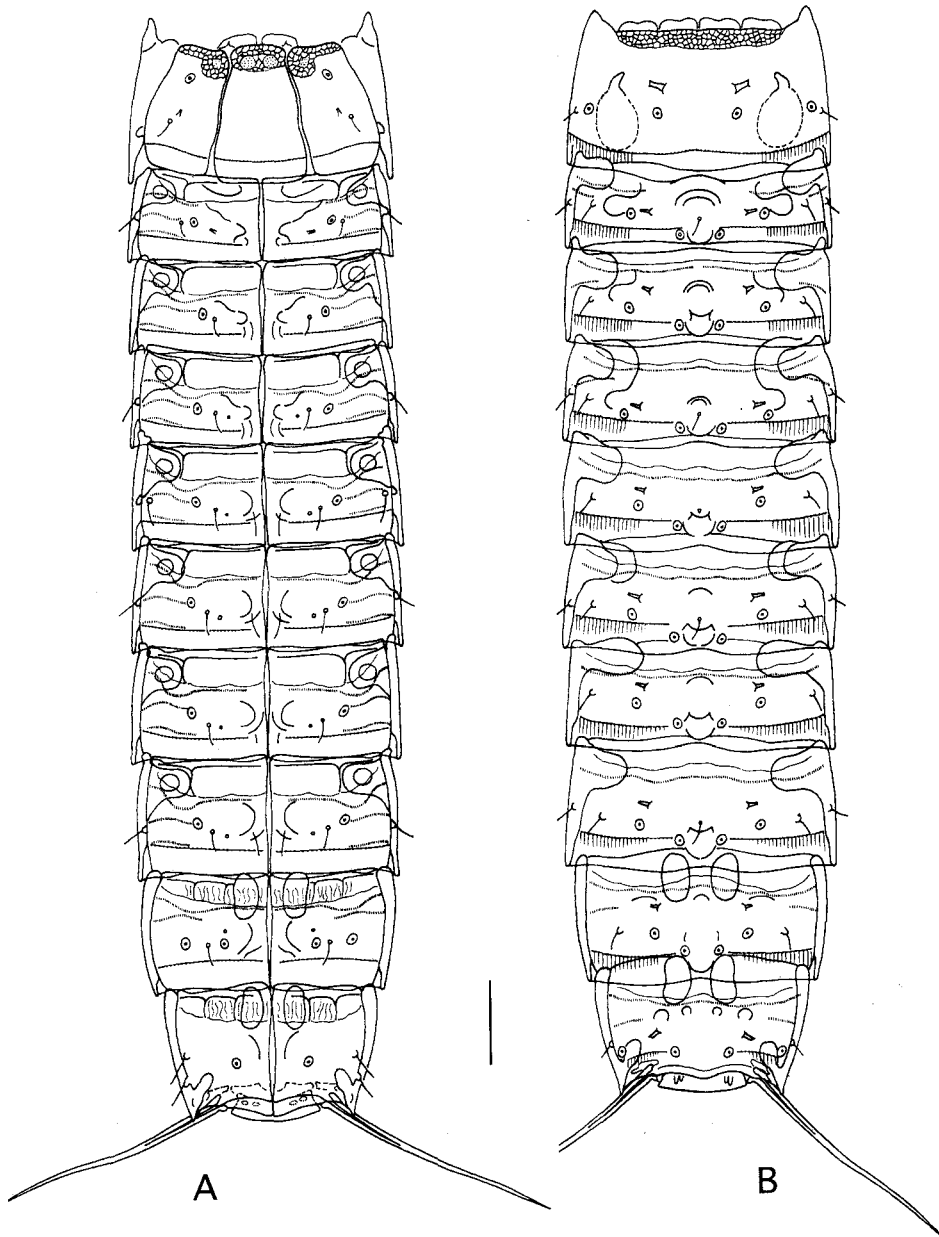


Fig. 2. Adult female of *P. tubuliferus* from Japan (AVA JAP-1.2).  
A-ventral view; B-dorsal view. Bar 50  $\mu$ m.

Segment 5. L-5 54  $\mu$ m; MSW-5 128  $\mu$ m; similar to segment 4 except for the absence of anterior middorsal projection, middorsal seta, one pair of dorsolateral setae, and lateral setae; in contrast to segment 4, subventral sensory spots are situated laterally to subventral seta.

Segment 6. L-6 60  $\mu$ m; MSW-6 132  $\mu$ m; similar to segment 5 except for the presence of middorsal seta, additional pair of dorsolateral setae, and lateral setae.

Segment 7. L-7 63  $\mu$ m; MSW-7 134  $\mu$ m; similar to segment 5 except for the presence of a pair

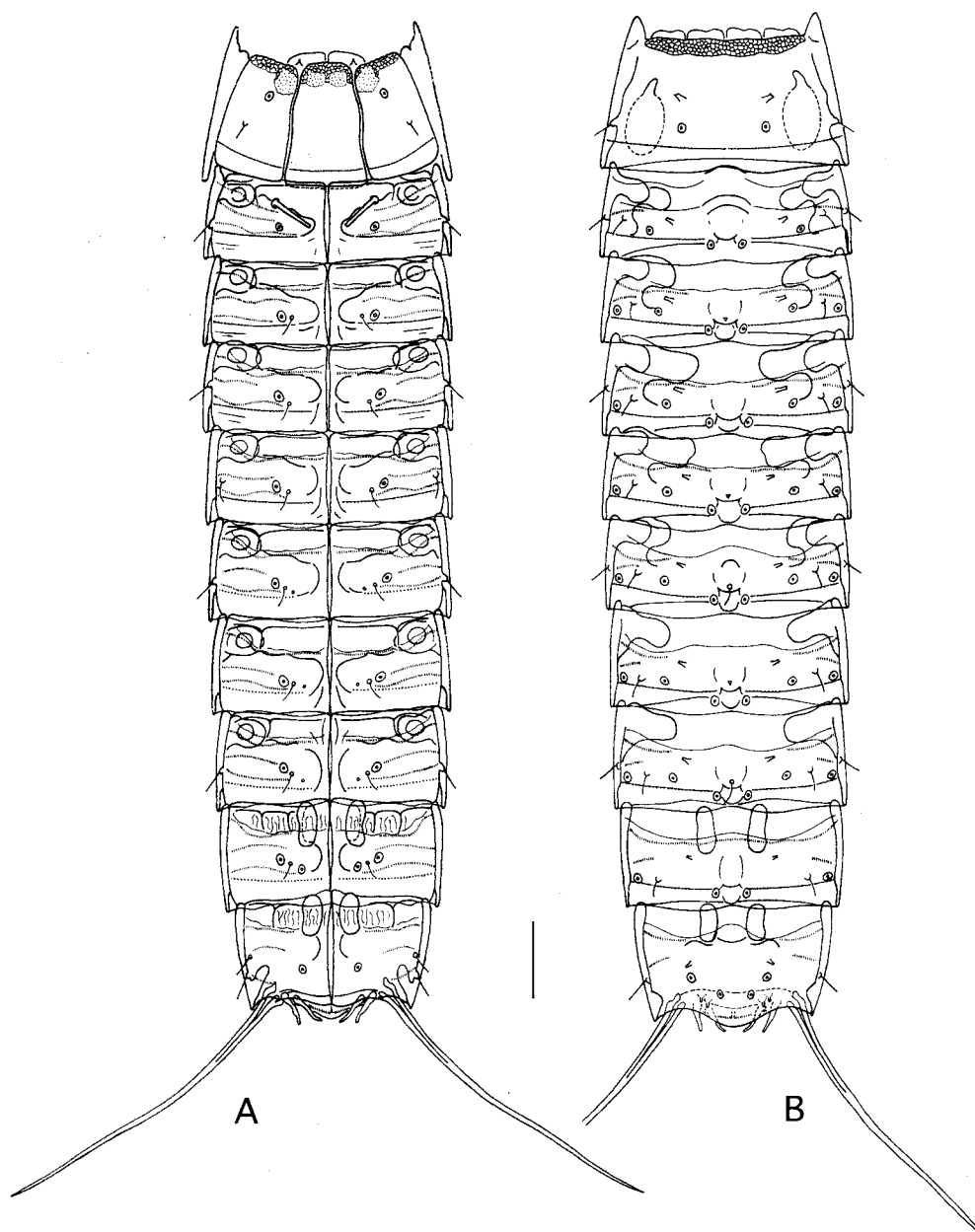


Fig. 3. Adult male of *P. tubuliferus* from Japan (CM JAP-22.8).  
A-ventral view; B-dorsal view. Bar 50  $\mu\text{m}$ .

of ventrolateral setae.

Segment 8. L-8 66  $\mu\text{m}$ ; MSW-8 136  $\mu\text{m}$ ; similar to segment 6.

Segment 9. L-9 68  $\mu\text{m}$ ; MSW-9 135  $\mu\text{m}$ ; similar to segment 5.

Segment 10. L-10 72  $\mu\text{m}$ ; MSW-10 132  $\mu\text{m}$ ; similar to segment 8.

Segment 11. L-11 72  $\mu\text{m}$ ; MSW-11 124  $\mu\text{m}$ ; similar to segment 9 except for the presence of a pair of separated anteriomesial thickenings of ventral pachycycli ("Mittelwuelste"), additional pair

of subventral sensory spots lateral to subventral seta, well-developed area of reticulate cuticle at anterior margin of sternal and tergal plates; and except for the absence of characteristic peg-and-socket tergal-sternal articulations.

Segment 12. L-12 77  $\mu\text{m}$ ; SW 106  $\mu\text{m}$ ; tergal plate with two pairs of rounded subdorsal muscular scar and a pair of tubular muscular scars, with a pair of subdorsal sensory spots and a pair of dorsolateral sensory spots, with a pair of dorsolateral setae; tergal and sternal plates with wide anterior area of reticular cuticle, with a pair of subventral sensory spots and a pair of ventrolateral setae; sternites with characteristically enlarged posterolateral pachycycli; lateral plates with a pair of lateral setae; segment with a pair of separated anteromesial thickenings of ventral pachycycli; posterior margins of tergal and sternal plates slightly concave.

Segment 13. L-13 18  $\mu\text{m}$ ; MSW-13 52  $\mu\text{m}$ ; tergal plate trapezoidal, with two pairs of subdorsal tubular papillae with floscular tip; the paired basements of tubular papillae well visible from the ventral side of segment 13 through use of Nomarsky optics (see Fig. 4); posterior margin of sternal plates minutely fringed; LTS 154  $\mu\text{m}$ , 24.1% of TL, thin and slightly curved.

(2) Adult male (CM JAP-22.8) (Fig. 3 A-B)-TL 572  $\mu\text{m}$ ; MSW-7 126  $\mu\text{m}$ , 22% of TL; SW 103  $\mu\text{m}$ , 18% of TL; LTS 196  $\mu\text{m}$ , 34.3% of TL.

Segment 1. Head is withdrawn.

Segment 2. Neck is composed of 4 dorsal placids with concave anterior margin and 2 ventral placids with even anterior margin. Cuticular fold between segments 2 and 3 with two sharp horns protruding laterally of anterior margin of midsternal plate of segment 3.

Segment 3. 1st trunk segment; L-3 94  $\mu\text{m}$ ; anterior tergal margin slightly wavy, with wide submarginal area of reticulate cuticle, width of anterior margin 104  $\mu\text{m}$ ; horn-like protrusion well developed, prominently elongated, slightly blunt; tergal plate with a pair of subdorsal tubular cuticular scars, a pair of subdorsal sensory spots posterior to cuticular scars, a pair of large dorsolateral muscular scars, and a pair of dorsolateral setae; midsternal plate trapezoidal, 72  $\mu\text{m}$  long, width of anterior margin 28, width of posterior margin 48  $\mu\text{m}$  (anterior/posterior=58%); midsternal plate with oval-shaped area of thin cuticle with two separated spots; anterior margin of midsternal plate slightly protruding beyond anteromesial margins of episternal plates; episternal plate with large anteromesial areas of thin cuticle with reticulate microrelief at anterior margin and rounded mesial zone of thin cuticle; each episternal plate with a ventrolateral seta and sensory spot near thin cuticle area.

Segment 4. L-4 50  $\mu\text{m}$ ; MSW-4 120  $\mu\text{m}$ ; tergal plate with minute middorsal element, never protruding beyond posterior margin of tergite; tergite with a pair of middorsal sensory spots near middorsal element, a pair of subdorsal cuticular scars, a pair of subdorsal sensory spots, and two pairs of dorsolateral setae; anterior margin of tergal plate with blunt anterior middorsal projection, slightly protruding beyond posterior margin of segment 3; posterior middorsal protrusion underdeveloped and never protruding beyond posterior margin of tergite; with characteristic peg-and-socket tergal-sternal articulation, with thick midventral and anteroventral pachycycli; sternal plates with a pair of adhesive tubes, 25  $\mu\text{m}$  long, and with a pair of subventral sensory spots; posterior margin of sternal plates slightly concave laterally; with a pair of lateral setae on tergal plate next two articulation site; tergal and sternal plates with series of parallel transverse cuticular ridges of minute fringe, extending mesially from lateral margins, but only visible through use of SEM.

Segment 5. L-5 50  $\mu\text{m}$ ; MSW-5 123  $\mu\text{m}$ ; similar to segment 4 except for the presence of a pair of subventral seta and a pair of dorsolateral sensory spots lateral to dorsolateral seta, and except for the absence of anterior middorsal projection, adhesive tubes, lateral setae, and a pair of dorsolateral setae.

Segment 6. L-6 52  $\mu\text{m}$ ; MSW-6 126  $\mu\text{m}$ ; similar to segment 5 except for the presence of additional pair of dorsolateral setae and a pair of lateral setae.

Segment 7. L-7 56  $\mu\text{m}$ ; MSW-7 126  $\mu\text{m}$ ; similar to segment 5 except for the presence of a pair



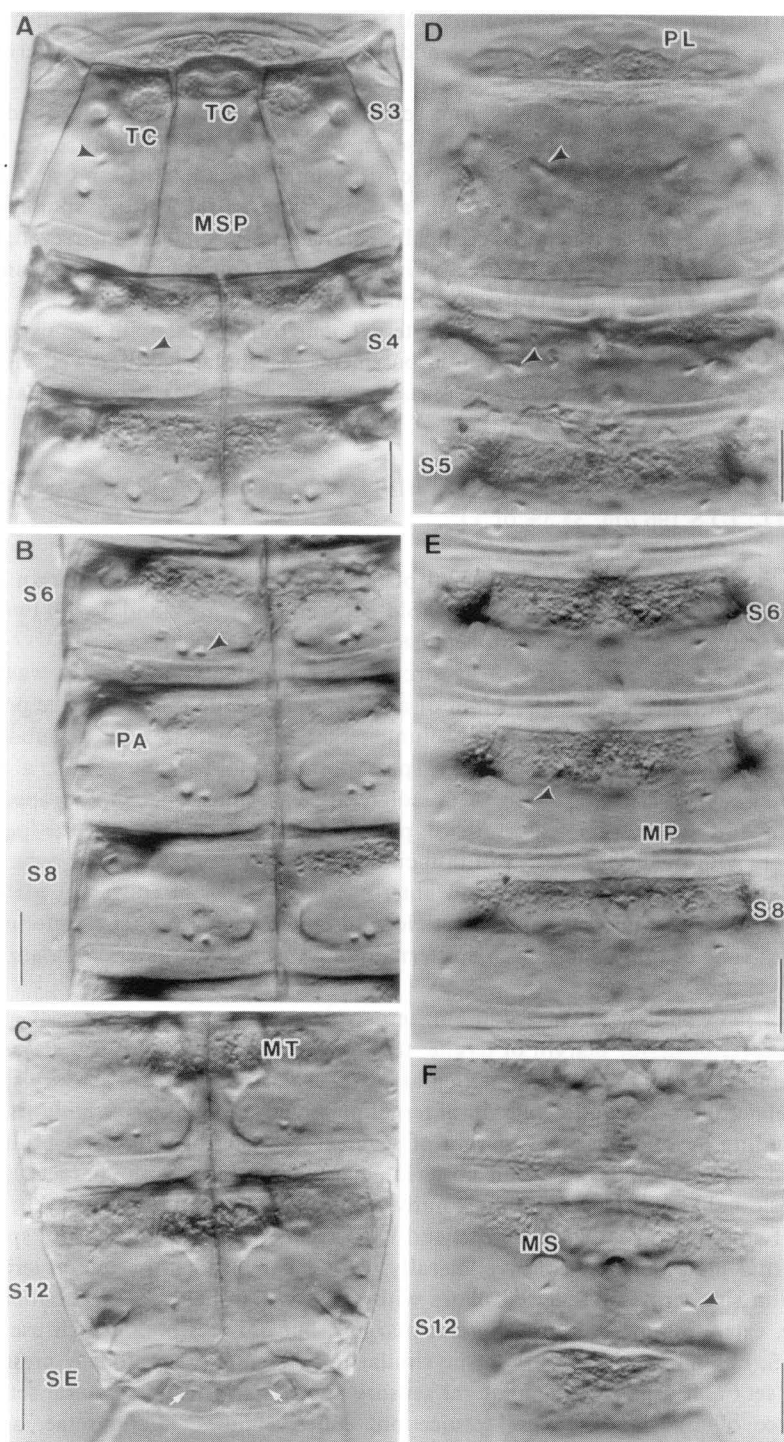


Fig. 4. Adult female of *P. tubuliferus* from Japan (AVA JAP-1.1); Image with Nomarsky optics. A-C-ventral view; D-F-dorsal view. A,D-segments 3-5; B,E-segments 6-8; C,F-segments 11-13. Black arrowheads indicate muscular scars (MS). White arrowheads indicate basements of tubular papillae. Bar 25  $\mu$ m.

of ventrolateral setae.

Segment 8. L-8 59  $\mu\text{m}$ ; MSW-8 124  $\mu\text{m}$ ; similar to segment 6 except for the presence of middorsal seta and a pair of minute subventral cuticular scars mesially to subventral seta.

Segment 9. L-9 58  $\mu\text{m}$ ; MSW-9 120  $\mu\text{m}$ ; similar to segment 7 except for the presence of minute subventral cuticular scar mesial to subventral seta and except for the absence of a pair of ventrolateral setae.

Segment 10. L-10 58  $\mu\text{m}$ ; MSW-10 117  $\mu\text{m}$ ; similar to segment 8.

Segment 11. L-11 64  $\mu\text{m}$ ; MSW-11 118  $\mu\text{m}$ ; similar to segment 9 except for the presence of a pair of separated anteriomesial thickenings of ventral pachycycli ("Mittelwuelste"), additional pair of subventral sensory spots mesial to subventral seta, well-developed area of reticulate cuticle at anterior margin of sternal and tergal plates; and except for the absence of characteristic peg-and-socket tergal-sternal articulations, and a pair of subdorsal sensory spots mesial to dorsolateral seta.

Segment 12. L-12 64  $\mu\text{m}$ ; SW 103  $\mu\text{m}$ ; tergal plate with a pair of rounded subdorsal muscular scars and a pair of tubular muscular scars, with a pair of subdorsal sensory spots and a pair of dorsolateral sensory spots, with a pair of dorsolateral setae; tergal and sternal plates with wide anterior area of reticular cuticle, with a pair of subventral sensory spots and a pair of ventrolateral setae; sternites with characteristically enlarged posterolateral pachycycli; lateral plates with a pair of lateral setae; segment with a pair of separated anteromesial thickenings of ventral pachycycli; posterior margins of tergal and sternal plates concave; two pairs of penile spines between segments 12 and 13, PS-1 22, PS-2 20  $\mu\text{m}$ .

Segment 13. L-13 16  $\mu\text{m}$ ; MSW-13 46  $\mu\text{m}$ ; tergal plate trapezoidal, with two pairs of subdorsal tubular papillae with floscular anterior margin; the paired basements of tubular papillae well visible from the ventral side of segment 13 through use of Nomarsky optics; posterior margin of sternal plates minutely fringed; LTS 196  $\mu\text{m}$ , 34.3% of TL, thin and slightly curved.

The morphometric measurements for 5 females and 5 males from the same locality are presented in Table 1.

#### SEM observation

Some new ultrastructural details common for both females and males of *P. tubuliferus* have been found for the first time using the SEM (Figs. 5-7). A sclerotized transverse cuticular fold bearing two sharp horns is situated between ventral placids and anterior margin of sternal plates of the first trunk segment (Fig. 7 A). The horns are minute triangular elements, 2.5  $\mu\text{m}$  long, which protrude laterally from the anterior margin of midsternal plate. The anterior margin of the tergal plate of segment 4 has a blunt middorsal projection slightly protruding beyond posterior margin of anteriormost tergite (Fig. 6). Sternites and tergites bear transverse series of parallel cuticular ridges that are especially distinct near the lateral margins of sternal plates and alongside the anterior margin of tergal plates (Figs. 5, 6 B, 7 B-C). The series consisted of 1 to 4 ridges of minute fringe extending mesially from lateral margins to midventral and middorsal lines (Figs. 5, 6 B, 7 B-C). A tergal plate of segment 13 bears paired tubular papillae, 1.7  $\mu\text{m}$  long, with diameter 0.9  $\mu\text{m}$  at the top and 1.5  $\mu\text{m}$  at the basement (Figs. 7 C-D). Papillae show characteristic floscular appearance and possess a distal circlet of 9 rounded petals surrounding a simple central pore (Fig. 7 D). In the middle of the pore, there is a single free cilium (Fig. 7 D).

Based on the new morphological details described in *P. tubuliferus* from both localities, the Sea of Japan and the Pacific coast of Japan, we propose a new diagnosis of the species.

*Pycnophyes tubuliferus* Adrianov, 1989

Diagnosis. TL 510-650  $\mu\text{m}$ ; LTS in females 120-155  $\mu\text{m}$  (22-24%), in males 160-185  $\mu\text{m}$  (26-31%); 4 dorsal PL with concave anterior margin and 2 ventral PL with even anterior margin; HP elongated, slightly blunt; anterior margin of first tergal plate even or slightly wavy corresponding to dorsal PL; first tergite with wide submarginal area of RC; MSP with oval-shaped area of TC with two separated spots of thinner TC; anterior margin of MSP about 55-76% of posterior margin;

Table 1. Body measurements ( $\mu\text{m}$ ) for paratypes of *Pycnophyes tubuliferus*

Character (Abbr.)	Range			MEAN $\pm$ Standard deviation			Coefficient of variability		
	Male	Female	ALL	Male	Female	ALL	Male	Female	ALL
Number	5	5	10	5	5	10	5	5	10
TL	516.4–605.6	510.0–612.0	510.0–612.0	571.2 $\pm$ 34.2	553.4 $\pm$ 43.8	562.3 $\pm$ 38.2	5.989	7.915	6.799
SW	115.7–131.8	122.1–128.6	115.7–131.8	124.1 $\pm$ 5.8	123.4 $\pm$ 2.9	123.8 $\pm$ 4.4	4.706	2.329	3.517
MSW-7	141.4–167.1	138.2–163.9	138.2–167.1	153.0 $\pm$ 9.5	149.1 $\pm$ 9.5	151.1 $\pm$ 9.2	6.232	6.393	6.101
LTS	160.7–183.2	138.2–154.3	138.2–183.2	171.0 $\pm$ 10.0	148.5 $\pm$ 6.2	159.8 $\pm$ 14.2	5.854	4.164	8.900
L-3	86.8– 96.4	83.6– 96.4	83.6– 96.4	91.9 $\pm$ 3.7	90.6 $\pm$ 4.8	91.3 $\pm$ 4.1	3.987	5.260	4.454
L-4	54.6– 57.9	51.4– 61.1	51.4– 61.1	56.6 $\pm$ 1.8	55.3 $\pm$ 3.5	55.9 $\pm$ 2.7	3.112	6.369	4.846
L-5	57.9– 61.1	57.8– 64.3	57.9– 64.3	59.1 $\pm$ 1.8	61.1 $\pm$ 2.3	60.1 $\pm$ 2.2	2.977	3.722	3.609
L-6	61.1– 64.3	61.1– 67.5	61.1– 67.5	61.7 $\pm$ 1.4	63.6 $\pm$ 2.7	62.7 $\pm$ 2.3	2.329	4.226	3.626
L-7	61.1– 67.5	64.3– 70.7	61.1– 70.7	63.0 $\pm$ 2.9	66.2 $\pm$ 2.9	64.6 $\pm$ 3.2	4.563	4.342	4.947
L-8	61.1– 67.5	64.3– 70.7	61.1– 70.7	63.0 $\pm$ 2.9	66.9 $\pm$ 2.7	64.9 $\pm$ 3.3	4.563	4.022	5.113
L-9	61.1– 67.5	64.3– 70.7	61.1– 70.7	64.3 $\pm$ 2.3	67.5 $\pm$ 2.3	65.9 $\pm$ 2.7	3.536	3.367	4.146
L-10	64.3– 73.9	64.3– 70.7	64.3– 73.9	66.9 $\pm$ 4.2	68.8 $\pm$ 2.9	67.8 $\pm$ 3.5	6.268	4.180	5.216
L-11	67.5– 77.1	67.5– 77.1	67.5– 77.1	72.0 $\pm$ 3.7	72.6 $\pm$ 3.7	72.3 $\pm$ 3.5	5.090	5.045	4.801
L-12	70.7– 80.4	70.7– 80.4	70.7– 80.4	74.6 $\pm$ 7.1	77.1 $\pm$ 4.5	75.9 $\pm$ 4.1	4.722	5.893	5.360
L-13	22.5– 28.9	22.5– 28.9	22.5– 28.9	25.1 $\pm$ 2.7	25.1 $\pm$ 2.7	25.1 $\pm$ 2.5	10.726	10.726	10.113

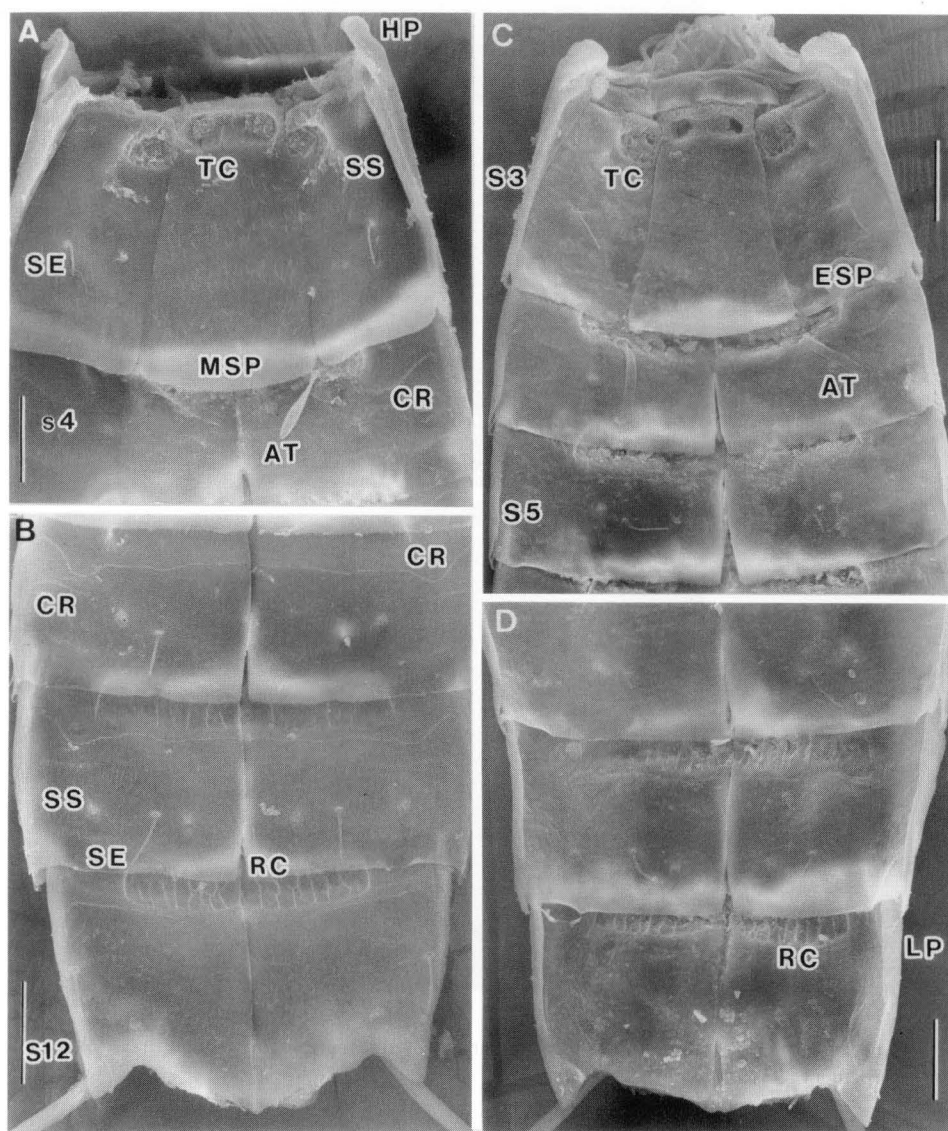


Fig. 5. *Pycnophyes tubuliferus*. SEM photographs of adult males; ventral view.

A-B—from Japan; C-D—from Korea.

A—segments 3-4; B,D—segments 11-13; C—segments 3-5.

Bar 25  $\mu$ m.

ESP with large anteromesial TC with reticulate appearance; MP underdeveloped, never protruding beyond posterior margin of tergal plate; segment 4 with AP slightly protruding beyond or even with posterior margin of previous tergite; tergal and sternal plates of segments 4-12 with more or less prominent transverse series of parallel CR of minute fringe; segments 4-12 with well developed peg-and-socket tergal-sternal articulations of PA; posterior margin of sternal plates on segments 4-12 slightly concave laterally; MT on segments 11-12, not adjacent at ventral midline; segment 12 with dorsolateral, ventrolateral and lateral SE; tergal plate of segment 13 with 4 large paired papillae

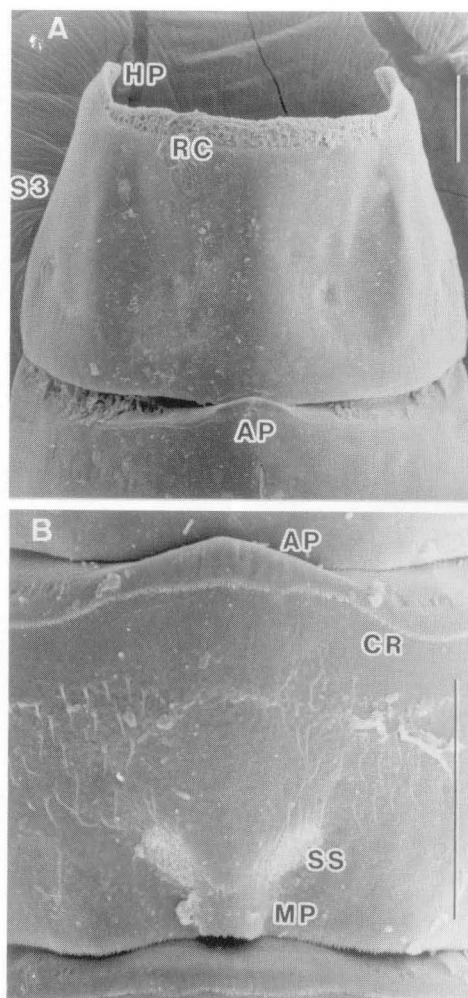


Fig. 6. *Pycnophyes tubuliferus* from Japan. SEM photographs of male; dorsal view. A—segments 3-4; B—segment 4.

with floscular appearance (TP).

### Discussion

Until now, *Pycnophyes tubuliferus* has been found only in the west coast of the Sea of Japan. This species was first described from the Peter the Great Bay ( $42^{\circ}54.3' \text{ N}$ ,  $132^{\circ}44.2' \text{ E}$ ) and then found in many localities alongside the Russian and the Korean coastlines of the Sea of Japan (Adrianov, 1989; Adrianov and Malakhov, 1999) (Fig. 1). The species is the most common species in shallow water of the west Sea of Japan and usually inhabits muddy biotopes at a depth of 5-30 m (Adrianov and Malakhov, 1999).

The specimens of *Pycnophyes* from the Tanabe Bay can be identified as *P. tubuliferus*, based on the main taxonomic characters of the species, including a double-spotted area of thin cuticle on the midsternal plate, shape of the midsternal plate, shape of thin cuticle areas on the episternal

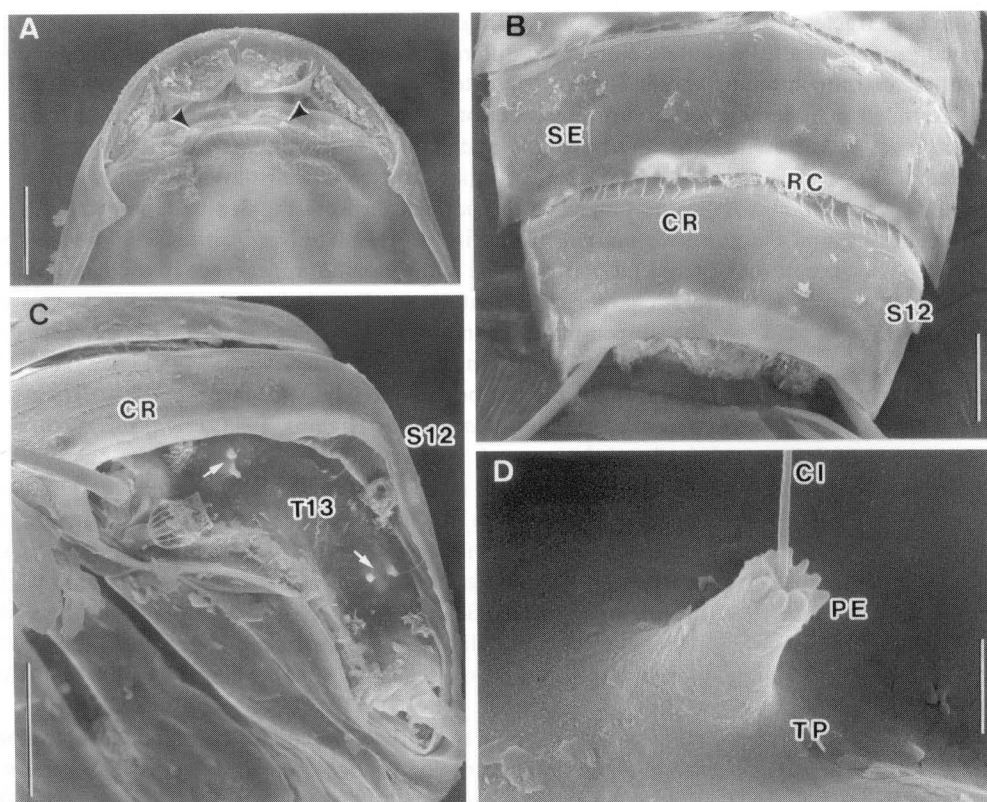


Fig. 7. SEM photographs of *P. tubuliferus* from Japan.

A—segment 3, ventral view;

B—segments 11–12, dorsal view;

C—tergal plate of segment 13, view from posterior;

D—floscular papilla.

Black arrowheads indicate minute horns between ventral placids.

White arrows indicate paired tubular papillae.

Bar A–C 25  $\mu$ m, D 1  $\mu$ m.

plates, paired papillae on the posteriormost tergal plate, characteristic shape of pachycycli and a number of setae on segment 12. At the same time, there are some morphological differences between representatives of these two populations from the west Sea of Japan and the Pacific coast of Japan. The specimens from the latter locality differ from the former in having slightly wavy anterior margin of the first tergal plate, the presence of much developed anterior middorsal projection on the tergal plate of segment 4, and the presence of additional dorsolateral setae on segments 4, 6 and 8. Specimens from the Sea of Japan are characterized by more even anterior margin of the first tergal plate and less developed anterior middorsal projection on segment 4. In contrast to the specimens from the Tanabe Bay, *P. tubuliferus* from the Sea of Japan possesses less-developed transverse cuticular ridges of fringe on trunk segments and has no additional dorsolateral setae on segments 4, 6 and 8. Specimens of *P. tubuliferus* from the Russian and the Korean coasts are very similar to each other and no distinct differences can be found except for more developed areas of reticulate cuticle on the sternal plates in specimens from Korea (Adrianov and Malakhov, 1999). Based on the present SEM observation, we suggest that all these differences may be variations of main



taxonomic characters in representatives of the single species but composed of two populations separated geographically, because the observed differences mentioned above are neither autapomorphic characters nor unique combinations of characters practical for taxonomy.

A rounded middorsal projection on the anterior margin of segment 4 is a unique character of *P. tubuliferus*, unknown in other pycnophyids and it may be considered as an autapomorphic character of the species (Fig. 6). Nevertheless, this character is well developed only within the Japanese population, and is less developed in the population from the Sea of Japan where it still not protrudes beyond the posterior margin of the first tergal plate (see Adrianov and Malakhov, 1999). The presence of four paired tubular papillae with floscular appearance in the middle of the posteriormost tergite is another characteristic feature of *P. tubuliferus* (Fig. 7 C). Several *Pycnophyes* species have well-developed papillae on the tergal plate of segment 13 (Zelinka, 1928; Higgins, 1983; Higgins and Kristensen, 1988; Adrianov, 1995; Adrianov and Malakhov, 1999). But most of these species have papillae only on the posterior margin (*P. greenlandicus* Higgins et Kristensen, 1988; *P. carinatus* Zelinka, 1928; *P. emarginatus* Higgins, 1983; *P. galtsovae* Adrianov in Adrianov et Malakhov, 1999; *P. spitsbergenensis* Adrianov, 1995). A few species have pairs of papillae in both the middle area and the posterior margin of the tergite (*P. cryopygus* Higgins et Kristensen, 1988; *P. newzealandiensis* Adrianov in Adrianov et Malakhov, 1999; *P. schornikovi* Adrianov in Adrianov et Malakhov, 1999). The only species with 4 large paired papillae situated in the middle of the posteriormost tergite is *P. furugelmi* Adrianov in Adrianov et Malakhov, 1999 also described from the Peter the Great Bay in the Sea of Japan. This species is well distinguished by the presence of well-developed middorsal tooth-like spines on segments 3–12 and by the absence of anteromesial thickenings of ventral pachycycli on segments 11–12 (Adrianov and Malakhov, 1999).

Based on TEM and SEM researches, the floscular papillae, flosculi and sensory spots have been considered as homologous sensory-glandular organs for probable chemoreception (Adrianov *et al.*, 1989; Nebelsick, 1992; Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994). Floscular papillae of *P. tubuliferus* with a circlet of 9 petals are very similar to the typical flosculi with 8–10 petals described only in adults of *Kinorhynchus yushini* Adrianov, 1989 (see Adrianov, 1989). These structures are very common only for juvenile stages of *Pycnophyes* and *Kinorhynchus* but always develop into multipapillate sensory spots in adult stages (Adrianov *et al.*, 1989; Neuhaus, 1993; Adrianov and Malakhov, 1994) and the typical flosculi have never been described in adult *Pycnophyes*.

Only a few species of *Pycnophyes* possess prominent transverse cuticular ridges of fringe, though the pattern is the most common for representatives of the genus *Kinorhynchus* (*P. longicornis* Higgins, 1983; *P. flaveolus* Brown in Adrianov et Malakhov, 1999; and *P. corrugatus* Higgins, 1983) (see Higgins, 1983; Adrianov and Malakhov, 1999). *Pycnophyes tubuliferus* is the only north-pacific species of the genus that possesses this character.

Most kinorhynch species have been described from only a single locality in the world and only a few species have been known from several localities in different aquatories (Higgins, 1983; Adrianov and Malakhov, 1999). Because of this, the comprehensive zoogeographical analysis still has never been proposed for the taxon. *P. tubuliferus* has been known from at least three separated localities (Russia, Korea and Japan) and is very abundant in shallow mud at a depth of about 35 m alongside the west coast line of the Sea of Japan and around the mainland of Japan. This species is thought to be a good subject for a future zoogeographical analysis that requires to study a larger number of specimens to exclude differences due to variations within a population or ageing.

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